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AN ARTIFICIAL STABILIZED COMPOSITION OF CALCIUM PHOSPHATE PHASES PARTICULARLY ADAPTED FOR SUPPORTING BONE CELL ACTIVITY

FIELD OF THE INVENTION

5   This invention relates to bioactive artificial stabilized sintered compositions of calcium phosphate phases which are capable of supporting bone cell activity thereon. This invention has applications in medical diagnostics for the assessment of normal and abnormal bone cell activity as well as for medical therapeutics including bone and dental tissue replacement

10   and repair as well as for *ex vivo* bone graft tissue engineering.

BACKGROUND OF THE INVENTION

Bone is a complex mineralizing system composed of an inorganic or mineral phase, an organic matrix phase, and water. The inorganic mineral

15   phase is composed of crystalline calcium phosphate salts while the organic matrix phase consists mostly of collagen and other noncollagenous proteins. Calcification of bone depends on the close association between the organic and inorganic phases to produce a mineralized tissue.

20   The process of bone growth is regulated to meet both structural and functional requirements. The cells involved in the processes of bone formation, maintenance, and resorption are osteoblasts, osteocytes, and osteoclasts. Osteoblasts synthesize the organic matrix, osteoid, of bone which after calcium phosphate crystal growth and collagen assembly

25   becomes mineralized. Osteocytes regulate the flux of calcium and phosphate between the bone mineral and the extracellular fluid. Osteoclasts function to resorb bone and are essential in the process of bone remodelling. Disturbing the natural balance of bone formation and resorption leads to various bone disorders. Increased osteoclast activity has been demonstrated to lead to

30   bone disease characterized by a decrease in bone density such as that seen in

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osteoporosis, osteitis fibrosa and in Paget's disease. All of these diseases are a result of increased bone resorption.

5   In order to understand the mechanisms involved which regulate bone cell functioning, it is important to be able to assess the normal function of bone cells and also the degree of perturbation of this activity in various bone diseases. This will lead to the identification of drugs targeted to restore abnormal bone cell activity back to within normal levels. Together with the identification of the etiology of abnormal and normal bone cell activity and

10 the assessment of this activity, is the desire and need to develop compositions and methods for the treatment of abnormal bone cell activity, as a result of disease, surgical removal or physiological trauma all of which lead to bone tissue loss. Therapeutics which provide for the replacement and repair of bone tissue, such as with the use of bone implants, are highly

15 desired.

    Several research groups have developed methods to directly observe the activity of isolated osteoclasts *in vitro*. Osteoclasts, isolated from bone marrow cell populations, have been cultured on thin slices of natural

20 materials such as sperm whale dentine (Boyde et al Brit. Dent. J. 156, 216, 1984) or bone (Chambers et al J. Cell Sci. 66, 383, 1984). The latter group have been able to show that this resorptive activity is not possessed by other cells of the mononuclear phagocyte series (Chambers & Horton, Calcif Tissue Int. 36, 556, 1984). More recent attempts to use other cell culture

25 techniques to study osteoclast lineage have still had to rely on the use of cortical bone slices (Amano et al. and Kerby et al J. Bone & Min. Res. 7(3)) for which the quantitation of resorptive activity relies upon either two dimensional analysis of resorption pit areas of variable depth or stereo mapping of the resorption volume. Such techniques provide at best an

30 accuracy of approximately 50% when assessing resorption of relatively thick

substrata. In addition these analysis techniques are also very time consuming and require highly specialized equipment and training. Furthermore, the preparation and subsequent examination of bone or dentine slices is neither an easy nor practical method for the assessment of osteoclast
5   activity.

The use of artificial calcium phosphate preparations as substrata for osteoclast cultures has also met with little success. Jones et al (Anat. Embryol 170, 247, 1984) reported that osteoclasts resorb synthetic apatites
10   *in vitro* but failed to provide experimental evidence to support this observation. Shimizu et al (Bone and Mineral 6, 261, 1989) have reported that isolated osteoclasts resorb only devitalized bone surfaces and not synthetic calcium hydroxyapatite. These results would indicate that functional osteoclasts are difficult to culture *in vitro*.
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Several groups have also attempted to provide compositions suitable for the therapeutic replacement of bone tissue. US Patent No. 4,871,578 discloses a process for the formation of a non-porous smooth coating of hydroxyapatite suitable for implant use. US Patent No. 4,983,182 discloses a
20   ceramic implant which comprises a sintered body of zirconia and a coating of α-TCP and zirconia, or hydroxyapatite and zirconia. US Patent No. 4,988,362 discloses a composition for the fusion of a bioceramic to another bioceramic. US Patent No. 4,990,163 discloses a coating used for the production of bioceramics which consist of α-TCP and β-TCP. Although
25   these different compositions may be used as biocompatible coatings for implants and the like, none of these compositions have been demonstrated to be suitable for the culture of both active osteoclasts and osteoblasts in a reliable and reproducible manner such to allow for the quantitative assessment of the specific activity of osteoclast resorption and osteoblast
30   secretion of bone matrix. Furthermore, none of the prior compositions

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developed, can be manipulated to reliably produce a range of films, thicker coatings and bulk ceramic pieces which share a common composition and morphology which leads to similar bioactive performance *in vivo* and *in vitro*.

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Applicant's published international PCT patent application WO94/26872 describes a sintering process for forming thin films of calcium phosphate phases on which bone cell function occurs. This is the first thin layer of synthetic material on which osteoclasts can exhibit extended activity

10 and on which osteoblasts may secrete bone matrix. As described in that application, a variety of factors should be considered in providing a thin film with a desired ratio of hydroxyapatite to tricalcium phosphate. Such parameters include:

1)   amounts of reagents for preparing the sol-gel hydroxyapatite
15   substance;

2)   rate of combination of reagents;

3)   duration and rate of mixing when making the sol- gel;

4)   rates and methods of precipitation and separation;

5)   process environmental conditions during the manufacture of the
20   sol-gel;

6)   velocity of removal of the substrate from the sol-gel in dip
         coating a film thereon;

7)   sintering temperature;

8)   sintering in a controlled atmosphere such inert gas, a vacuum
25   or an atmosphere with water vapour present.

9)   the nature of the substrate, with quartz being a preferred
         embodiment to create a transparent substrate coated with
         stabilized calcium phosphate phases.

30   It was suggested in this earlier PCT patent application, that in order

to obtain a broad range in ratios of hydroxyapatite to tricalcium phosphate on quartz substrates, many of these parameters needed to be considered in order to achieve the ratios of 10:90 through to 90:10. The suggested sintering temperatures in an air atmosphere were from approximately 800°C

5   to approximately 1100°. It was established that at 800°C the film was predominantly hydroxyapatite. A sintering temperature of about 900°C provided ratios of about 70:30. At 1000°C, the ratio was about 10:90 and at 1100°C the film was predominantly tricalcium phosphate. It was also suggested that sintering in a vacuum at 1000°C produced a ratio of

10 approximately 66:34. It has now been found that the preferred ratios are from 50:50 to 20:80. The optimum ratio is approximately 333:666. To achieve these ratios, consideration can be given to several of the above factors. However, it is desirable to minimize the variability in several of the above factors and to achieve the desired ratios for optimum film

15 compositions in an exacting reproducible manner. Surprisingly, this film is stable in the presence of various aqueous media, even though α-tricalcium phosphate is supposed to be soluble in water.

Applicants have discovered that the presence of stabilizing entities can

20 stabilize the composition and prevent its degradation in physiological fluids. Hence, disappearance of calcium phosphate entities from a film, coating or bulk ceramic piece of this composition, is substantially due to the activity of the osteoclasts and not due to a dissolution process. The stabilized artificial bioactive composition is the first such composition which supports both

25 osteoclast and osteoblast activity and which allows for the reliable assessment of the physiological activities of both cell types as well as for the development of both diagnostic and therapeutic strategies. The stabilizing entities stabilize the α-tricalcium phosphate within the calcium phosphate phases formed during sintering to provide for a stable form of α-tricalcium

30 phosphate which does not degrade in physiological fluids and which forms

calcium phosphate phases having a cell compatible morphology which supports and encourages bone cell activity thereon.

SUMMARY OF THE INVENTION

5   The present invention provides a stabilized composition which provides for a wide variety of diagnostic and therapeutic applications. The stabilized composition, in accordance with an aspect of the invention, can be used to provide a range of thin films, coatings, powders and bulk ceramic pieces which share a common surface globular microporisity and an internal

10   microporosity. In addition, the bulk ceramics also can have a macroporosity within the structure in order to provide an articifial three dimensional bone tissue similar to that found *in vivo*. The composition, made in any form, encourages the activity of bone cells cultured thereon and also allows for the development of *ex vivo* engineered artificial bone tissues to use as bone

15   grafts.

In accordance with an aspect of the invention there is provided a bioactive artificial sintered composition for providing a morphology capable of consistently supporting bone cell activity thereon, the composition comprising stabilized calcium phosphate phases developed by the conversion

20   of a hydroxyapatite substance in the presence of stabilizing entities at sintering temperatures into insolubilized and stabilized tricalcium phosphate.

According to another aspect of the present invention is a process for stabilizing an artificial sintered composition of calcium phosphate phases

25   having a morphology suitable for supporting bone cell activity thereon, the process comprising converting an hydroxyapatite substance, into primarily alpha tricalcium phosphate by sintering, and providing stabilizing entities which stabilize and insolubilize the formed alpha tricalcium phosphate within the phosphate phases.

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According to yet another aspect of the present invention is a sintered artificial microporous polycrystalline structure for supporting bone cell activity, the structure comprising sintered stabilized calcium phosphate phases having a globular surface morphology of loosely interconnected rounded granules with interconnected micropores in the structure.

According to yet another aspect of the present invention is an implantable calcified bone matrix comprising:

a) a structure for supporting the matrix;

b) a layer of stabilized calcium phosphate phases developed by the conversion of a hydroxyapatite substance in the presence of stabilizing entities at sintering temperatures into tricalcium phosphate where the stabilizing entities insolubilize and stabilize the calcium phosphate phases;

c) a boundary layer deposited by osteoblasts cultured on the layer of stabilized calcium phosphate phases; and

d) a mineralizing collagenous matrix secreted by such cultured osteoblasts.

According to another aspect of the present invention is a method for the culturing of functional bone cells, the method comprising :

- applying a suspension of bone cells in physiological media to an artificial sintered film of stabilized calcium phosphate phases on a substrate comprising stabilized and insolubilized alpha tricalcium phosphate complexes.

According to another aspect of the present invention is a kit for monitoring and quantifying the activity of bone cells, the kit comprising;

- a substrate having a sintered film of calcium phosphate phases containing stabilized and insolubilized alpha tricalcium phosphate,

- a multiwell bone cell culture device adhered to the substrate.

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According to another aspect of the invention is a method for the *ex vivo* tissue engineering of mineralized collagenous matrix, the method comprising the steps of:

      - providing an artificial stabilized composition having a globular surface morphology of loosely interconnected rounded granules with interconnected micropores,

      - applying a suspension of osteoblasts in physiological media on the composition,

      - incubating the mineralized collagenous bone matrix selected by the osteoblasts from the culture; and

      - implanting the isolated collagenous bone matrix in a patient.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a predominance area diagram showing the effect of CaO activity on the stabilities of hydroxyapatite and tricalcium phosphate.

Figure 2 is a plot showing the phases of calcium phosphate entities formed in the presence of stabilizing silicon entities from the conversion of the hydroxyapatite substance of the present invention.

Figure 3 is a plot showing the effect of CaO/Al_2O_3 on the activity of CaO.

Figure 4 is a plot showing the effect of CaO/TiO_2 and CaO/B_2O_3 ratios on the activity of CaO.

Figure 5 comprises graphs (a), (b) and (c) which show results of energy dispersive x-ray spectroscopy (a) at the interface of the composition with the substrate; (b) just above the interface and (c) at the top of the film.

Figure 6 is a cross-sectional SEM of mineralized collagenous bone matrix deposited on a stabilized thin film composition of the present invention by active osteoblasts.

Figure 7 (a) is a photograph showing the deposition of florescent calcified bone matrix produced by osteoblasts cultured on the stabilized

composition.

Figure 7 (b) is a photograph showing a control in which no osteoblasts are cultured on the stabilized thin film composition and no fluorescent calcified bone matrix is visualized.

5   Figure 8 is a SEM of osteoclast resorption pits on bulk three dimensional solid ceramics composed of artificial bioactive stabilized compositions.

Figure 9 is a SEM of osteoclast resorption pits on thin films of artificial bioactive stabilized compositions.

Figure 10 is a cross-sectional TEM magnified micrograph of a thin layer

10   of the artificial stabilized composition on a quartz substrate showing the morphology.

Figure 11 is a SEM micrograph of the stabilized composition applied as a thin film showing the surface microporous structure.

Figure 12(a)is a SEM micrograph of a commercial sintered

15   hydroxyapatite in the absence of stabilizing entities.

Figure 12(b) is a SEM micrograph of commercial sintered hydroxyapatite in the presence of silicon stabilizing entities.

Figure 13 is a SEM of an osteoclast on natural bone showing a natural resorption pit.

20   Figure 14 is a SEM micrograph illustrating the globular surface morphology of a bulk ceramic made by the process of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The composition of calcium phosphate substance is that provided in

25   accordance with that described in the applicant's co-pending published PCT application WO94/26872. This process provides on a consistent basis, a thin film of calcium phosphate phases which are within the desired range of 50:50 to 20:80 for the ratio of hydroxyapatite to α-tricalcium phosphate. It has now been found that the presence of stabilizing entities significantly and

30   unexpectantly stabilizes the α-TCP within the calcium phosphate phases to

provide a bioactive composition which supports and encourages the activity of both osteoblasts and osteoclasts and which allows for the quantification of such activity in a reproducible manner, and provides for the development of diagnostic and therapeutic strategies for bone tissue loss.

It is to be understood that the term "stabilized" refers to the calcium phosphate phases formed upon conversion of the hydroxyapatite which maintain a consistent crystallographic and chemical structure when placed in ambient conditions or in a physiological environment *in vivo* or *in vitro*. It is also to be understood that the term "bioactive" refers to the ability to support osteoblastic bone growth over and throughout structures substantially or exclusively made of the present composition and simultaneously promote natural controlled extracellular resorption of the composition by osteoclasts, while avoiding non-specific chemical and/or cellular dissolution and/or degradation, in a process closely resembling that of normal bone turnover. Such bioactivity being present during *in vitro* and *in vivo* uses of the materials where bone cells are present. By the term "calcium phosphate phases", it is intended to include the various calcium phosphate species in the sintered product such as hydroxyapatite α-TCP, β-TCP, calcium octophosphate, tetracalcium phosphate and dicalcium phosphate.

It was initially thought that for supporting bone cell activity *in vitro*, the suitable calcium phosphate product was to be pure or essentially pure hydroxyapatite and was understood to be the calcium phosphate entity of choice in making a film. It has now been determined that materials which are predominantly of hydroxyapatite do not encourage normal function of osteoclasts and osteoblasts and, in actual fact, in the presence of osteoclasts, very little activity can be observed. It was found, however, that by providing a mixture of calcium phosphate phases which include hydroxyapatite and α-tricalcium phosphate, the degree of resorption is encouraged through a broad

range where the film predominantly of α-tricalcium phosphate provides the highest degree of resorption, whereas a film predominantly of hydroxyapatite provides a negligible degree of resorption. It is this realization, with respect to the presence of α-tricalcium phosphate that partially suggests why the presently

5   developed calcium phosphate materials encourage functional properties in bone cells being cultured on such materials. This aspect, in providing stabilized calcium phosphate phases in the form of a thin film which permits, for example, transmittance of light or light reflection, allows one to carry out diagnostic procedures to evaluate several functional properties of bone cells being cultured

10   on such films.

Surprisingly, it has been found that standardizing the preparation of the hydroxyapatite sol-gel substance and selecting a very specific range of sintering temperatures, not only achieves the desired ratios but also reveals that the

15   optimum composition is formed by conversion of hydroxyapatite as prepared by the sol-gel process to α-tricalcium phosphate. Little or no β-tricalcium phosphate has been detected in these preferred optimized film compositions. There is no need to prepare mixtures of hydroxyapatite and α-tricalcium phosphate preparations of the individual phases. Instead, the technique as

20   described in published PCT application WO 94/26872 is sufficient in preparing a sol-gel hydroxyapatite substance. The chemical reaction for making such a hydroxyapatite substance in a medium of elevated pH is as follows:

$$5Ca(NO_3)_2 + 3NH_4H_2PO_4 + 7NH_4OH$$
$$\rightarrow Ca_5(PO_4)_3OH + 10NH_4NO_3 + 6H_2O$$
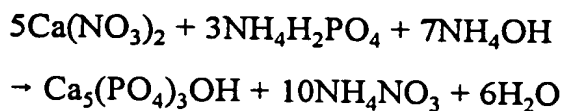
25   The starting solutions consist of aqueous solutions in which the reactants are fully dissolved and which can be well mixed. The hydroxyapatite forms as fine particles in suspension, the size of which is shown by light scattering experiments to grow from an average of about 0.3 μm to over 1 μm when aging the sol-gel substance for 24 hours after preparation.

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The hydroxyapatite substance is stable in neutral and/or alkaline media. Preferably the reaction medium is brought to an elevated pH usually in the range of about 12. A first phosphate solution is added drop by drop into a second calcium solution to prevent the formation of tetracalcium

5   monohydrogen triphosphate thereby obtaining a product of the desired hydroxyapatite. The sol-gel substance can be filtered, dried as a powder, calcined and fired in an alumina crucible at 1000°C, to form calcium hydroxyapatite phases which are stable under normal conditions of atmospheric humidity. The conversion of this phase at sintering temperatures of over

10   1200°C is into primarily α-tricalcium phosphate with smaller amounts of some other phases also being formed such as β-TCP, calcium octophosphate, tetracalcium phosphate or dicalcium phosphate. It is to be understood by those skilled in the art other "contaminating" materials may also be formed in the sintered stabilized calcium phosphate phases. Such materials may also be added

15   to the hydroxyapatite substance before sintering. The presence or addition of such contaminants preferably does not affect the composition and the morphology of the stabilized composition in any manner which will affect the support of bone cell activity thereon.

20   With respect to the sintering process, it has also been found that sintering of the dried film of hydroxyapatite substance may be carried out in a standard type of high temperature oven without any need to control the atmosphere in the oven. When a new oven is used or an oven contaminated by previous use for other purposes, it is preferred to cycle the oven through the

25   sintering temperature range several times while the oven is empty. Such pre-conditioning of the oven removes any volatiles and prepares it for use. No additional steps are required. Ambient air may be present in the oven during the break-in period and during normal use for sintering coated substrates where the presence of ambient air does not hamper the process and results in

30   producing consistent results for the desired ratio. Under these conditions, the

sintering temperature may range from 920°C up to 1100°C in providing the desired ratios of 50:50 up to 20:80 in the presence of a quartz substrate. It has been found that as the temperature increases, the conversion of hydroxyapatite into α-tricalcium phosphate is also increased. At sintering temperatures in the

5   range of 920°C up to 950°C the ratio may vary from 50:50 towards 333:666. At selected sintering temperatures in the range of 950°C to 1000° the ratio is approximately 333:666. Increasing the temperature beyond a 1000°C and up to 1100°C further increases the conversion and produces compositions having ratios in the range of 333:666 to 20:80. The preferred sintering temperature is

10   approximately 975°C where the ratio of 333:666 is achieved.

        The conversion of the hydroxyapatite to tricalcium phosphate occurs via the reaction;

$$2Ca_5(OH)(PO_4)_3 \rightarrow 3Ca_3(PO_4)_2 + CaO + H_2O$$

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the degree of conversion at any temperature being sensitive to the partial pressure of water in the surrounding atmosphere and to factors which modify the concentration of CaO.

20   The nature of the tricalcium phosphate which is formed is of significance. For non-stoichiometric hydroxyapatite with a Ca/P ratio of 1.5 - 1.60 (Nakamura, Thermochimica Acta, Vol. 165, 1990), and for many commercially supplied hydroxyapatite powders (Aldrich Chem Co.), β-tricalcium phosphate is often formed when the powder is heated to 1100°C and

25   then cooled to temperatures below 1000°C. β-TCP is a stable, insoluble compound which appears in nature as the mineral Whitlockite. In the conversion of the sol-gel derived hydroxyapatite substance formed from aqueous solutions as described herein, and in calcium hydroxyapatite powders formed from alternate precipitation reactions, it is found that the formation of

30   α-tricalcium phosphate is enhanced at temperatures below 1000°C in the

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presence of stabilizing entities. In the development of calcium phosphate based coatings, α-TCP has not been a great subject of attention because of its degradation in physiological fluids due to its relatively high solubility and from the fact that it only results from the high temperature conversion of pure

5   hydroxyapatite at temperatures of over 1250°C.

From the conversion equation, it is expected that any factor which controls the activity of CaO in the system will modify both the temperature and the reversibility of the hydroxyapatite conversion. The addition of a stabilizing

10   entity such as SiO_2 is believed to react with CaO by the reaction:

$$CaO + SiO_2 \rightarrow CaSiO_3$$
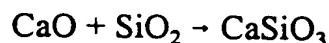
thereby driving the conversion to lower temperatures. This reaction should be complete for 1 mol SiO_2 per 1 mol CaO produced in the reaction. Other reactions to form different silicates with other CaO/SiO_2 ratios may be possible.

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When CaO is removed by the action of the silica to form calcium silicates, the temperature at which the TCP phases form is reduced to temperatures consistent with the data from the conversion of the prepared hydroxyapatite composition as seen in Figure 1. The addition of silicon entities

20   drives the conversion line to the right, that is, to lower temperatures with the formation of primarily α-TCP.

The proposed mechanism by which silica plays a direct role in encouraging the formation of the alpha tricalcium phosphate compared to other

25   phases such as beta tricalcium phosphate is that the silicon entities enter the hydroxyapatite crystal structure and stabilizes the alpha phase with respect to beta. It has now also been demonstrated, in accordance with a preferred embodiment, that the nature of the starting hydroxyapatite substance and the manner in which silica is added is of importance. When silica in the form of a

30   powder is added to a commercial pure hydroxyapatite powder, and co-milled to

promote mixing, the conversion product observed at high sintering temperatures of over 1000°C was β-TCP. In contrast, powders prepared according to the present invention with silica added as a metal-organic solution, converted to primarily a stabilized alpha tricalcium phosphate phase which was

5   retained at low temperature as shown in Figure 2 at the 950°C line. This conversion is not reversible. At high temperature, the doped powders show a reduction in the conversion temperature from over 1200°C for pure powders to about 950°C for silica doped powders. As noted, this development is believed to be due to the formation of calcium silicates, whereby the stabilized resulting

10 phase composition is retained on cooling to low temperature.

One reason why powders with stabilizing entities prepared in accordance with the present invention have reproducible and stable phase compositions with a desired surface morphology and an internal microporous structure is that

15 the hydroxyapatite substance is originally prepared in the sol-gel process as very fine particles. Addition of stabilizing entities such as silicon entities in the form of a metallorganic solution allows each of these particles to be in intimate contact with a layer of silicon entities resulting in thorough mixing. On sintering, the silica is in close proximity to the CaO released in the conversion

20 reaction. It is proposed that the formation of insoluble calcium silicate entities at the surface of each particle limits the reversibility of the reaction and plays a role in preventing the solubility of alpha tricalcium phosphate in aqueous physiological media.

25   In a similar manner to silica, titanium, aluminum and boron were predicted to reduce the conversion temperature and thus can be used as stabilizers, ie. dopants. Figures 3 and 4 illustrate the temperature reduction with the formation of CaO/Al/Ti/Ba complexes. These metals may be used to remove the CaO from the hydroxyapatite and result in a stabilized α-TCP. The

30 factors important for the selection of stabilizer (dopant) and of the compound

by which it is dispersed are: (a) it needs to interact with formed CaO which forms a stable calcium compound, (b) it must be capable of being dispersed uniformly throughout the sol-gel substance preferably in a manner which surrounds the outer surfaces of the newly formed particles, (c) it should not

5   stabilize undesirable phases within the calcium phosphate system, and (d) it must be non-toxic when integrated for biological applications. Stabilizing entities suitable for use in the present invention are those which form oxides, preferably metal oxides. Preferred metal oxides are those selected which produce the desired composition and morphology such as oxides of aluminum,

10   zirconium, germanium, chromium, vanadium and niobium, and are more preferably selected from silicon and titanium oxides. Mixtures of such stabilizing entities may also prove useful.

       Sintering is done in the presence of stabilizing entities. The stabilizing

15   entities can be provided by virtue of diffusion from a substrate through the hydroxyapatite substance during the sintering process or by the addition of stabilizing entities to the hydroxyapatite substance before sintering. Whether by diffusion or by addition, the stabilizing entities are provided in an amount sufficient to stabilize the calcium phosphate phases which are in the form of

20   thin films, powders, thicker coatings, bulk ceramic pieces and in bulk ceramic pieces having an internal macroporosity formed therein. Preferably for the support and encouragement of bone cell activity, it is the resultant unique bioactive surface morphology and internal microporous structure which are reproducible and which are a function of the presence of the stabilizing entities

25   during the sintering process.

       Depending on its proposed use, the composition can be provided as various structures such as in the form of thin films for diagnostics or as thicker coatings to be used on bone or dental implants. Herein, thin films can be

30   described as those having a thickness of 0.1 microns to 5 microns, thicker

coatings are those having a thickness above 5 microns designed to be applied to other substrates. Bulk ceramic pieces refer to larger three dimensional structures which are functionally independent of a substrate.

5   A preferred embodiment is provided by sintering a film of hydroxyapatite substance on the face of a quartz substrate. The quartz substrate provides a sufficient source of silicon entities which can diffuse throughout the calcium phosphate phases and produce a sufficient silicon entity content. As shown in Figure 5 (a), the interface of the composition with the substrate,

10   Figure 5 (b) just above the interface, and Figure 5 (c) of the top of the film, silicon entities are available throughout the film composition. During the sintering period, silicon entities are released from the surface of the quartz and diffuse through the surface of the hydroxyapatite substance layer. During the conversion of the hydroxyapatite into α-tricalcium phosphate within the

15   preferred range for the ratios of hydroxyapatite to α-tricalcium phosphate, the silicon entities react with the CaO to form calcium silicates which form a stable complex with the α-tricalcium phosphate. It is understood by those skilled in the art that other substrates or additives which upon sintering release stabilizing entities into the developing calcium phosphate phases, may also be used in

20   accordance with the present invention. The stabilizing entities may be selected from metal and non-metal oxides of, for example, silicon, aluminum, zirconium, boron, titanium germanium, chromium, vanadium, niobium and mixtures thereof. Substrates containing or made of aluminum, zirconium, boron, titanium and various mixtures of these components may be suitable for

25   providing the source of stabilizing entities.

        The thin film as provided on a suitable support, in accordance with this invention, significantly advances the study and understanding of bone cell functional properties. The make-up of the stabilized film, as provided in

30   accordance with this invention, permits the culture of various types of bone

cells thereon. The surface make-up may be adjusted to encourage a significant degree of resorption of the calcium phosphate entities of the film material through to a negligible degree of resorption of the calcium phosphate entities in the study of osteoclast activity. Similarly, osteoblast activity may be studied by

5   detecting a build up of calcified bone matrix. The ability to provide the material in a film which is sufficiently thin that resorption of the entities by osteoclasts can be detected by the disappearance of resorbed calcium phosphate entities provides a simple inexpensive format for analysis compared to the prior art techniques. The film make-up as made in accordance with this invention,

10   supports the biological function of bone cells. The benefit in providing the film on a transparent supporting substrate, such as quartz or glass, lends to easy evaluation techniques of the diagnostic process including automated machine reading.

15   Ideally the film thickness is greater than 0.1 micron because it has been found that at film thicknesses less than 0.1 microns it is difficult to obtain uniform film coverage, free from discrete voids. As to the upper thickness limit for the film, it can be of any desired thickness depending upon its end use. As will be discussed, the degree of resorption may be detected by light

20   transmittance, which preferably requires a film less than 10 microns in thickness. The substrate is of quartz which readily withstands the required sintering temperatures and has the desired degree of transparency to permit light transmittance tests to determine the extent of resorption of calcium phosphate entities from the film material.

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The developed thin films may be used in kits and the like to provide for assessment of bone cell activity. The film may be embodied in the form of a "kit" comprising quartz substrates, pre-coated with an adherent calcium phosphate thin film, which may be used in a cell culture vessel (possibly a 24-

30   well optionally sterilized multi-well plate i.e. of approximately 15 mm

diameter) as a system suitable for the culture of mixed bone cell populations. The device is simple and relies on only routine laboratory equipment and techniques for use, is suitable for quantitative analysis, and is inexpensive to fabricate but strong enough to withstand normal levels of handling and may be

5   packaged in lots, of (for example) 24 samples in a plastic presentation box. The thin film surfaces have a defined and reproducible chemistry and are mechanically strong enough to withstand transport when used with an appropriate packing material.

10   In each case the culture conditions may be such that osteoclasts, in either mononuclear or multinucleate form could be expected to survive in a functional state and resorb the artificial calcium phosphate of the film. Similarly, osteoblast are also capable of actively secreting calcified bone matrix under such culture conditions.

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These substrates may be used to assess the resorptive activity of osteoclasts and monitor the change in this level of resorptive activity either as a result of a disease process or the inclusion, in the culture medium, of an agent such as a drug which would influence, either directly or indirectly, osteoclastic

20   resorptive activity. The substrates are also suitable for the culture of active osteoblasts in order to observe and assess the secretion of bone matrix thereon as well as use the deposited mineralized matrix for *in vivo* transplantation. As seen in Figure 6, mineralized collagenous matrix 10 is deposited by cultured osteoblasts on the surface of the stabilized thin film 12 as provided on a quartz

25   substrate 14. A well integrated boundary layer 16 resembling a cement line is shown and which is similar to the same type of cement lines formed by osteoblasts *in vivo* at the interface between new bone and old bone. This clearly suggests that the pressure stabilized composition allows for physiological osteoblast activity further supporting the role of the stabilized

30   composition as an important bone remodelling product.

The device may be used as a means of quantifying the resorptive activity of osteoclasts or build-up of bone-like material by the activity of osteoblasts. Such activity analysis may occur under continuous real-time monitoring, time-lapse intervals or end-point determination. The steps in establishing bone cell

5   activity are common to each of the above monitoring schedules in that bone cells (either animal or human) are cultured, in specific conditions, on one or more of the devices. The culture period is from several hours to many days and preferably from approximately 2 to 10 days (the optimum time is cell species and protocol dependent), during which time the extent of osteoclast activity

10   may be continuously monitored, periodically monitored, or simply not monitored on an on-going basis in favour of final-end-point determination. Similarly, osteoblast activity may be observed by determining extent of calcified bone matrix build-up. As is shown in Figure 7, a quartz disc coated with a stabilized film of the present invention and simultaneously cultured with

15   osteoblasts (a), is highly fluorescent indicating the presence of mineralized bone matrix. In contrast, a stabilized film coated on quartz in the presence of medium alone (b) shows no fluorescence. The amount of calcified bone matrix is directly proportional to the measurable fluorescence emitted. Tetracycline is a naturally fluorescent material. As the cells take up tetracycline, it is

20   metabolized and its metabolites are secreted and incorporated into the newly formed bone matrix. The tetracyline will only fluoresce upon its being metabolized by the osteoblasts. This demonstrates that osteoblasts actively secrete bone matrix on the stabilized composition.

25   Once the sol-gel hydroxyapatite substance is prepared, it may be applied as a thin film to the desired substrate in a variety of techniques. For example, the dip-coating method (C.J. Brinker et al., Fundamentals of Sol-Gel Dip Coating, Thin Solid Films, Vol. 201, No. 1, 97-108, 1991) consists of a series of processes: withdrawal of the substrate from a sol or solution at a constant

30   speed, drying the coated liquid film at a suitable temperature, and firing the film

to a final ceramic.

In spin-coating the sol-gel is dropped on a plate which is rotating at a speed sufficient to distribute the solution uniformly by centrifugal action.

5 Subsequent treatments are the same as those of dip coating.

It is appreciated that there are a variety of other techniques which may be used to apply a thin film of the sol-gel to the substrate. Other techniques include a spraying of the sol-gel, roller application of the sol-gel, spreading of

10 the sol-gel and painting of the sol-gel.

An alternative to coating discrete discs of a singular size is to coat an enlarged substrate with a film of the sol-gel. The entire film on the substrate is then sintered. A device, such as a grid, may then be applied over the film to

15 divide it into a plurality of discrete test zones.

In these various techniques of the sol-gel substance application, the thickness and quality (porosity, microstructure, crystalline state and uniformity) of formed films are affected by many factors. These include the physical

20 properties, composition and concentration of the starting sol, the cleanliness of the substrate surface, withdrawal speed of the substrate and the firing temperature. In general the thickness depends mainly on the withdrawal rate and sol viscosity for a dip coating process. Since heterogeneity in the sol is responsible for the formation of macropores and cracks, the coating operation

25 should be undertaken in a clean room to avoid particulate contamination of the sol. At the heat-treatment stage, high temperatures are required to develop the required microstructure and desired conversion of hydroxyapatite to α-tricalcium phosphate.

30 The purpose of applying the dip coating method to fabricate calcium

phosphate films is twofold: (a) to make films with required qualities (uniformity, thickness, porosity, etc.); and (b) to make translucent calcium phosphate films on transparent substrates for biological experiments.

5   It has also been found that the stabilized artificial composition of the present invention is suitable for the production of not only thin films and thicker coatings, but also powders and bulk ceramics. Ceramics are prepared from sintered powders prepared by the sol-gel method described herein with the addition of silica to create a desired stabilized hydroxyapatite/alpha tricalcium

10   phosphate phase mixture. In one embodiment the sintered powder is finely ground in an amount sufficient to produce a disc 0.5 - 1mm thick which is then mixed with a drop of retained sol-gel substance of the same dopant composition to create a damp powder which helps in keeping the particles together. The damp powder is uniaxillally pressed in a laboratory die at a pressure of about 5

15   tonnes/cm^2. The resultant bulk material shows good green strength and is fired at 1000°C for 1 hour in air. Such ceramics retain all of the same characteristics as the stabilized composition as used as a thin film, or coating. For SiO$_2$ stabilized compositions, X-ray diffraction showed little change in the phase composition between the initial powder and the final ceramic. The surface

20   topography as seen in Figure 8, in plan view, is strikingly similar to that of the composition coated on a quartz substrate illustrated in Figure 9. The resorptive capability of the osteoclasts on thin films and the bulk ceramics are very similar. Osteoclast resorption is observed as the presence of resorption pits 18 on the bulk ceramic, and are similar to resorption pits 18 witnessed on the thin

25   films (Figures 8 and 9).

        As recognized by one skilled in the art, larger ceramic bulk pieces could be formed by shaping of the ceramic to form bulk pieces for use in desired applications. The bulk pieces produced retain the desired stabilized calcium

30   phosphate phase composition as well as the microporous globular surface

morphology, and internal microporous structure both of which facilitate bone cell activity thereon.

A particular aspect of ceramic preparation for use in biological applications is the fabrication of ceramic pieces with a fine globular surface microporosity and internal microporosity which leads to bioactivity, and a larger macrostructure of pores of dimensions 50-1000 μm or more, within the internal structure. This encourages bone remodelling in a system more closely resembling physiological *in vivo* bone remodelling. Such macroporosity at the low end of the range being particularly suited to applications desiring rapid ingrowth of bone matrix, while macroporosity at the high end of the range allow cells to access the interior for uses such as for *ex vivo* tissue engineering production of bone grafts. Using powders doped with a stabilizing entities such as silica and sintered prior to use, porous ceramics can be made by mixing of such powders with styrene balls of a desired size. After pressing a dampened doped powder with styrene balls, then at a required pressure, the styrene is then removed by pyrolysis at temperatures of about 400°C - 600°C. The porous ceramic is then fired to 1000°C in the normal manner as previously described. This procedure results in the formation of a bulk ceramic having an external globular microporous structure, an underlying internal microporous structure and an internal macroporous structure allowing cells to migrate and function throughout the entire bulk ceramic unit.

It is to be understood by those skilled in the art that materials similar to styrene may be used to develop macroporosity within the ceramic structure. Other materials which are capable of pyrolysis at temperatures below the normal sintering temperatures are also useful to form the macroporous structure. The materials used should also not leave any toxic residues. It is also understood that other methods can also be used to form the macrostructure such as mechanical drilling of holes, the use of lasers or use of foaming agents.

Since the significant aspect of the present invention is the mixture of hydroxyapatite and alpha tricalcium phosphate phases coupled with the surface morphology created by doped powder formed from the sol-gel hydroxyapatite substance, one skilled in the art will understand that other methods of

5   fabricating films, coatings and bulk structures from this powder may also be used in accordance with the present invention. This includes the use of known techniques such as plasma or thermal spray or electrophoretic deposition.

With reference to Figure 10, a cross-sectional TEM micrograph shows a

10   gradient of layers in the form of the quartz substrate (a), the interface layer (b), which comprises small grains, and (c) the upper layer which includes the surface of the film which is comprised of small crystallites embedded in granules which provides the globular microporous structure. During the sintering process, silicon entities are released from the quartz (a) and which

15   diffuse through the hydroxyapatite as it is converted to α-tricalcium phosphate in forming the sintered thin layer. The interface layer (b) has a smaller crystalline structure than the surface which has larger polycrystalline granules of the calcium phosphate phases.

20   The morphology of the artificial sintered composition is unique and has not been previously reported or demonstrated. We have now discovered a surface morphology presenting a loosely interconnected globular structure of rounded granules having a microporous structure of interconnected pores. In accordance with a preferred aspect of this invention, the morphology

25   successfully supports cultures of functional osteoclasts and osteoblasts.

The surface morphology of the coating has a characteristic form involving a loosely interconnected globular structure resembling coral (Figure 11). The size of the granules varies from approximately 0.5-1μm in lateral

30   dimension. The coating is porous in the direction perpendicular to the substrate

with a larger planar density closer to the surface than near to the substrate. This morphology may allow for the percolation of liquid media and other physiological fluids within the coating. In contrast, the surface morphology of hydroxyapatite prepared from other coprecipitation methods, does not result in a microporous structure as provided by the present invention. As seen in Figures 12(a) and 12(b), the surface morphology of such prepared hydroxyapatite films in the absence (a) and presence (b) of stabilizing entities is not microporous as compared to the present composition seen in Figure 11. In addition, is has been reported that synthetic polycrystalline hydroxyapatite is not resorbed by osteoclasts (Shimizu, Bone and Minerology, Vol. 6, 1989).

The globular surface morphology is made up of rounded granules comparable in size to the aggregated deposits initially made by an osteoblast cell in the process which leads to bone formation. The present composition provides a surface morphology compatible with the type of morphology the cell expects to encounter *in vivo*. A typical osteoclast resorption pit is shown in Figure 13, in which the substrate is bone. As demonstrated in Figure 9, the resorption pits 18 formed by osteoclasts 20 on the present artificial composition are extremely similar to those seen on natural bone of Figure 13 which suggests that osteoclasts function similarly in both systems. This implies that the surface morphology of the artificial sintered composition is compatible with the type of morphology the cell expects to encounter *in vivo*.

The bulk microporosity of the stabilized composition may ensure that the calcium or phosphate ion concentrations near the surface of the artificial material are within the limits expected by the cell as encounter *in vivo* with natural bone which is made up of hydroxyapatite, collagen and other fibrous tissues. During osteoclast mediated extracellular dissolution processes which lead to resorption, this complex material leads to a particular local concentration of dissolution products. During the dissolution or resorption of

fully inorganic artificial hydroxyapatite or α-TCP, the resulting concentration limits for certain cellular behaviours are narrowly defined, for the cell to achieve an activity on artificial surfaces comparable to that on natural bone, means must be available to adjust the local concentration levels of elements

5 such as calcium. The porosity of the composition allows this to occur through flow or diffusion of media.

The stabilized bioactive artificial composition of the present invention provides a unique chemical composition together with a unique surface

10 morphology and internal microporous structure that has never previously been demonstrated. Compositions have not been previously reported which demonstrate consistent bone cell bioactivity *in vivo* and *in vitro* and which *in vitro* can be readily, accurately and repetitively quantified. The nature of the stabilized composition is versatile in that it can be provided in a powder, thin

15 film, thick coating, bulk ceramic piece or macroporous bulk ceramic piece. In each case, the unique surface morphology and internal microporosity is maintained as well as the stabilized calcium phosphate phase composition. As seen in Figure 14, the microporous surface morphology is maintained on a bulk ceramic prepared from the present stabilized composition.

20

The stabilized composition of the present invention is ideal for *in vitro* diagnostics in order to characterize abnormal bone cell functioning in a large-scale and automated manner. The stabilized artificial composition is also readily applicable as a coating for bone and dental implants to promote tissue

25 regeneration and repair. The structure of the composition is such that is it very similar and therefore compatible with bone tissues and cells *in vivo* which will avoid the problems of rejection of foreign materials.

The present composition has the required physical characteristics and

30 affinity/compatibility with *in vivo* hard tissues such that it can be used for a

wide variety of therapeutic applications such as for providing implants *in vivo* as well as for the regeneration and repair of bone tissue *in vivo* as for example in hip and knee replacements, fractures, and dental implants. The composition can also be used for various tissue engineering applications performed *ex vivo*

5   in order to provide artificially produced bone material which can then be transplanted as bone grafts *in vivo* for bone tissue replacement, regeneration and repair. Patients can provide the supply of osteoclasts and osteoblasts used to culture on the composition in order to lessen the chances of tissue rejection thereby producing fully compatible bone grafts. Alternatively, donor bone cells

10   may also be used for this purpose. Such grafts can be prepared for tissue replacement in the absence or presence of the bone cells used to produce the bone tissue. It is preferred, however, that cell-containing grafts be from autologous donors in order to minimize problems associated with tissue rejection. The stabilized composition, in powder form, may also be used for

15   medical therapeutics. Stabilized powders may be mixed and suspended within polymeric substances, compatible with tissues and non-toxic, and then applied *in vivo* for the filing of voids within bone tissues.

All of the applications in which the present composition can be used

20   have the advantage that both osteoclasts and osteoblasts function actively with the composition in any form thus providing for a bone tissue system much like that found *in vivo*. The artificial bioactive composition of the present invention promotes both osteoconduction and resorption so that normal tissue healing and regeneration can occur while simultaneously allowing the artificial material to

25   be resorbed in the process of normal bone tissue remodelling.

The following procedures exemplify aspects of the invention for providing a bioactive artificial sintered composition which has stabilized calcium phosphate entities therein and which also displays a unique

30   morphology capable of supporting bone cell activity thereon.

Procedure 1, Preparation of Hydroxyapatite Sol-Gel Substance

The following procedure is based on preparing sufficient sol-gel hydroxyapatite for manufacturing purposes. Solution A comprises a calcium nitrate tetrahydrate and Solution B comprises an ammonium dihydrogen

5   orthophosphate (mono basic). Solution A is mixed with Solution B to produce the desired sol-gel, Solution C. Solution A is prepared by adding 40 mls of doubly distilled water to 4.722 grams of calcium nitrate, $Ca(NO_3)_2$. The solution is stirred at moderate speed for sufficient time to dissolve all of the calcium nitrate which is normally in the range of 3 minutes. To this solution, 3

10   mls of ammonia hydroxide (NH_4OH) is added and stirred for approximately another 3 minutes. The pH of the solution is tested where a pH of about 12 is desired. To this solution is added 37 mls of double distilled water to provide a total solution volume of approximately 80 mls. The solution is stirred for another 7 minutes and covered.

15

Solution B is prepared by adding 60 mls of double distilled water to a 250 ml beaker containing 1.382 grams of $NH_4H_2PO_4$. The beaker is covered and stirred at moderate speed for 3 to 4 minutes until all $NH_4H_2PO_4$ is dissolved. To this solution is added 71 mls of NH_4OH and the beaker then

20   covered and stirring continued for approximately another 7 minutes. The pH of the solution is tested where a pH of about 12 is desired. To this is added another 61 mls of double distilled water and the beaker covered to provide a total solution volume of approximately 192 mls. The solution is then stirred for a further 7 minutes and covered.

25

The desired sol-gel is then prepared by combining Solution B with Solution A. All of Solution A is introduced to a 500 ml reagent bottle. Stirring is commenced at a moderate speed and Solution B introduced to the reagent bottle at a rate of approximately 256 mls per hour until all 192 ml of Solution B

30   is delivered into Solution A. An excess of Solution B may be used to

compensate for any solution which may remain in the 250 ml beaker or any tubing used in the transfer process. After completion of this addition and combination of Solution A with Solution B, the resultant Solution is continued to be stirred at moderate speed for approximately 23 to 24 hours. The resultant

5   sol-gel is inspected for any abnormal precipitation or agglomeration. If any abnormal precipitation or agglomeration has occurred, the solution must be discarded and preparation commenced again. The sol is then carefully transferred to another 500 ml reagent bottle so as to avoid any inclusion of particle agglomerations that may be present on the walls of the original reagent

10   bottle. Approximately 240 mls of Solution C, that is the resultant sol-gel, is delivered to a centrifuge bottle and centrifuged for 20 minutes at about 500 rpm at room temperature. Following centrifugation, 180 mls of supernatant is discarded without disturbing the sediments. The sediments are gently resuspended by mixing in a smooth rotating manner for about 30 minutes.

15   Viscosity of the sol-gel is then measured and preferably is between 20 to 60 cP. The sol-gel is then ready for dip coating of the selected substrate or for other applications.

Procedure 2 - Preparation of Silica Doped Hydroxyapatite Substance

20   A silica solution is prepared as follows. The amounts determined create approximately 0.168 g SiO_2/4ml solution. 4 mls of silica solution is added to 60 ml of centrifuged hydroxyapatite sol-gel substance created in procedure 1 and reacted with 0.168 g CaO produced in the conversion reaction.

25   Silicon Solution Components

Tetrapropyl orthosilicate $Si(OC_3H_7)_4$	7.32 gm
2-methoxyethanol $CH_3OCH_2CH_2OH$	34.5 gm

30   The silica solution is added to the hydroxyapatite substance prepared by

30

procedure 1, such that the concentration of SiO_2 is of the ratio 1M of SiO_2/1 mol CaO produced upon conversion during sintering.

5   **Procedure 3 - Preparation of Thin Film Formats**

Depending upon the nature of the substrate, the preparation of thin films can use either Procedure 1 or Procedure 2 for the preparation of the hydroxyapatite sol-gel substance. If the substrate contributes to the availability of the necessary stabilizing entities within the sintered film then Procedure 1

10   can be adopted. If the substrate does not provide for the availability of such stabilizing entities, then Procedure 2 with the dopant additives is required.

Before the application of the thin film to the substrate, the substrate needs to be thoroughly cleaned to ensure satisfactory film coverage. In the case

15   of quartz substrates, cleaning is achieved by placing the discs in a glass beaker and supplying chromic acid cleaning solution to the glass beaker to cover all discs. The beaker is then covered. The discs are then sonicated in a water bath for 1 hour. The acid is washed away using tap water for 20 minutes. The residual tap water is removed by three changes of doubly distilled water. After

20   the final change of double distilled water, every single disc is dried with lint-free towel and inspected for flaws in the quartz surface. Any residual particulate on the surface is removed as needed with compressed nitrogen or air. The discs are stored in covered trays in an aseptic environment. This method can be used to clean any type of quartz substrate.

25

The quartz disc substrate, or other substrate having an appropriate composition, is dipped in the sol-gel prepared by Procedure 1. The disc is grasped at the edges to avoid touching the surface. The disc is dipped in the sol, preferably by machine. The disc is removed from the sol at a prescribed

30   withdrawing velocity. The coating on one side of the disc is removed. The

coated substrate is then placed in a clean Petrie dish and covered and dried at room temperature. The film, as formed prior to sintering, should be uniform without cracks, clumps or voids. It is understood that the dip coating process as applied to a face of a disc, may also be applied to any other shape of substrate,

5   such as, a flat rectangular shaped substrate of quartz.

Procedure 4 - Preparation of Dry Hydroxyapatite Powder

The sol-gel substance prepared by procedure 1 or 2 is dried at 100°C for approximately 8 hours. The dried substance is then ground using a mortar and

10   pestle or any other mechanism which can grind and produce a fine powder. The powder can then be sintered following the standard sintering process, as described in Procedure 7, except that the powder is sintered in a crucible and then again ground after cooling. The same procedure can be followed for the preparation of a doped or stabilized hydroxyapatite.

15

Procedure 5 - Production of Bulk Ceramic Pieces

Ceramics (three-dimensional bulk pieces) have been created from silica doped hydroxyapatite powders according to the following. A doped sol-gel substance was produced according to procedure 2. Part of the sol-gel substance

20   was saved and the remainder filtered. The powder was dried at 120°C and ground to produce a fine powder. About 0.09 gm of powder was placed in a plastic dish. A glass dropper was used to form a drop of the initial sol-gel substance, which weighed about 0.055 gm when mixed with the powder. The sol was mixed with the powder to form a damp, but not wet paste. The damp

25   paste was packed into a stainless steel die of diameter 6.25 mm and pressed at 2 metric tonnes for 1 minute. The bulk piece was removed from the press, air dried, and fired in a covered alumina crucible according to Procedure 7. Surface morphology was very similar to that of the artificial sintered thin films as seen in Figure 14.

30

Procedure 6 - Preparation of Bulk Ceramic Pieces having a Macrostructure of Pores

Hydroxyapatite prepared powders which are doped with a stabilizing entity such as silica as in Procedure 2 and sintered at 1000°C, are mixed with

5   styrene balls of a desired size, dampening the powder with additional sol-gel substance, and pressing at a pressure of about 1 tonne/cm² so as not to extrude the styrene. Enhanced green strength is achieved in such pressed powder/styrene compacts using a damping mixture of retained sol and 2.5 wt % solution of polyvinyl alcohol. The styrene is removed by pyrolysis by heating

10   in air or oxygen to 550°C. The macroporous ceramic is then fired to 1000°C in the manner as described for normal sintering in Prodecure 7.

Procedure 7 - Sintering of the Hydroxyapatite Substance

The following sintering process may be carried out in standard

15   laboratory furnaces of various sizes, capable of operating at temperatures from ambient up to at least 1100°C, and designed to maintain accurate and stable internal temperatures, particularly between 800°C and 1100°C, such as Lindberg models 51744 or 894-Blue M. The components prepared by Procedures 3, 4, 5 or 6 are carefully transferred onto a standard ceramic plate

20   (as is common practice in the Lindberg oven). The ceramic plate is used as a carrier during the sintering process to facilitate easy loading and withdrawal of multiple substrates from the furnace. The furnace temperature is set to the temperature required to achieve the desired ratios of HA:α-TCP. Utilizing a programmable furnace such as the Lindberg model 894-Blue M, the furnace

25   may be programmed to hold the desired temperature, which will normally be selected from the range 920°C to 1100°C, for a maximum of one hour to ensure desired diffusion of the silicon entities through the developed gradient layers of hydroxyapatite and α-tricalcium phosphate. In the case of non-programmable furnaces, a separate timer may be used to warn the operator to

30   turn the furnace off at the end of the required sintering time at selected

temperature. The ceramic plate carrying the sintered substrates is removed at any time after the internal furnace temperature has cooled to an acceptable and safe touch-temperature of approximately 60°C. Individual substrates may then be stored or packaged for final use.

5

In accordance with this process, thin films and thicker coatings of hydroxyapatite/α-tricalcium phosphate can be produced on a consistent basis having the desired composition where variability in the various processing parameters have been minimized to ensure such consistency.

10

Although preferred embodiments of the invention are described herein in detail, it will be understood by those skilled in the art that variations may be made thereto without departing from the spirit of the invention or the scope of appended claims.